

**AMENDMENTS TO THE CLAIMS:**

Amend the claims as follows:

Claims 1-30. (Canceled)

31. (New) A recombinant protein having (a) luciferase activity (b) at least 60% identity to luciferase from *Photinus pyralis*, *Luciola mingrellica*, *Luciola cruciata*, *Luciola lateralis*, *Hotaria paroula*, *Pyrophorus plagiophthalmus*, *Lampyris noctiluca*, *Pyrocoelia nayako* or *Photinus pennsylanvanica*, and (c) being a mutated wild-type sequence wherein in the sequence of the recombinant protein, at least one of

- (a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase or to residue 216 of *Luciola mingrellica*, *luciola cruciata* or *Luciola lateralis* luciferase;
- (b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase or to residue 234 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase;
- (c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase or to residue 297 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase;
- (d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to residue 16 of *Luciola mingrellica*, or 17 of *Luciola cruciata* or *Luciola lateralis*;
- (e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to residue 37 of *Luciola mingrellica*, or 38 of *Luciola cruciata* or *Luciola*

*lateralis*;

(f) the amino acid residue corresponding to amino acid residue 105 of the *Photinus pyralis* luciferase or to residue 106 of *Luciola mingrellica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis* gene;

(g) amino acid residue corresponding to amino acid residue 234 of the *Photinus pyralis* luciferase or to residue 236 of *Luciola mingrellica*, *Luciola cruciata* or *luciola lateralis*;

(h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase or to residue 422 of *Luciferase mingrellica*, *Luciola cruciata* or *Luciola Lateralis*;

(i) amino acid residue corresponding to amino acid residue 310 of the *Photinus pyralis* luciferase or to residue 312 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola Lateralis*

is different to the amino acid which appears in the corresponding wild type sequence and wherein the protein has increased thermostability as compared to an enzyme having the amino acid of the corresponding wild-type luciferase at this position.

32. (New) A protein according to claim 31 which has the sequence of a wild-type luciferase except that more than one amino acid residue is different to that of the wild-type luciferase.

33. (New) A protein according to claim 32 wherein up to 50 amino acids are different to that of the wild type luciferase.

34. (New) A protein according to claim 31 which is a modified form of luciferase of *Photinus pyralis*, *Luciola mingrellica*, *Luciola cruciata* or *Luciola Lateralis* luciferase,

35. (New) A protein according to claim 31 having the sequence of luciferase of *Photinus pyralis*, wherein at least one of

- (a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase is other than threonine;
- (b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase is other than isoleucine;
- (c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase is other than phenylalanine;
- (d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase is other than phenylalanine;
- (e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase in other than leucine;
- (f) amino acid residue corresponding to amino acid residue 105 or the *Photinus pyralis* luciferase is other than alanine;
- (g) amino acid residue corresponding to amino acid residue 234 of the *Photinus pyralis* luciferase is other than aspartic acid;
- (h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase is other than serine;
- (i) amino acid residue corresponding to amino acid residue 310 of the *Photinus*

*pyralis* luciferase other than histidine,

36. (New) A protein according to claim 31 wherein protein has substantially the sequence of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* enzyme, and wherein at least one of

- (a) the amino acid residue corresponding to residue 216 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than glycine (for *Luciola mingrellica* based sequences) or aparagine (for *Luciola cruciata* or *Luciola Lateralis*) based sequences;
- (b) the amino acid residue corresponding to residue 234 *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than serine;
- (c) amino acid residue corresponding to residue 297 *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than leucine;
- (d) amino acid residue corresponding to amino acid 16 *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* is other than phenylalanine;
- (e) amino acid residue corresponding to residue 37 of *Luciola mingrellica*, or residue 3B of *Luciola cruciata* and *Luciola lateralis* other than lysine;
- (f) amino acid residue corresponding to amino acid residue 106 of *Luciola mingrellica*, 107 of *Luciola cruciata* or *Luciola lateralis*, or 108 of *luciola lateralis* gene is other than glycine;
- (g) amino acid residue corresponding to amino acid residue 236 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* is other than glycine;
- (h) amino acid residue corresponding to residue 422 of *Luciola mingrellica*,

*Luciola cruciata or Luciola lateralis* is other than threonine;

(i) amino acid residue corresponding to amino acid residue 312 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* is other than threonine (for *luciola mingrellica* based sequences) or valine (for *luciola cruciata* or *luciola lateralis*) based sequences.

37. (New) A protein according to claim 31 comprising a protein having luciferase activity and at least 60% identity to luciferase from *Photinus pyralis*, *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* wherein in the sequence of the protein, at least one of

(a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase and to residue 216 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase is mutated and is other than threonine in the case of *Photinus pyralis* luciferase; or

(b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase and to residue 234 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase is mutated and is other than isoleucine in the case of *Photinus pyralis* luciferase; or

(c) amino acid residue corresponding to residue 295 in *photinus pyralis* luciferase and to residue 297 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase is mutated and is for example, other than phenylalanine in the case of *Photinus pyralis* luciferase;

and the protein has increased thermostability as compared to *Photinus pyralis*,

*Luciola mingrellica, Luciola cruciata or Luciola lateralis* luciferase, on which the protein is based.

38. (New) A protein according to claim 31 wherein the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase and to residue 216 of *Luciola mingrellica, Luciola cruciata or Luciola lateralis* luciferase is alanine.

39. (New) A protein according to claim 31 wherein the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase and to residue 234 of *Luciola mingrellica, Luciola cruciata or Luciola lateralis* luciferase is alanine.

40. (New) A protein according to claim 31 which is a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase is leucine.

41. (New) A protein according to claim 31 wherein the amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to amino acid 16 in *Luciola* luciferase, is alanine.

42. (New) A protein according to claim 31 wherein the luciferase is a mutated luciferase of *Photinus pyralis* or a *Luciola* species where the amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to amino acid residue 37 in *Luciola mingrellica* or 38 of *luciola lateralis or cruciata* luciferase is alanine.

43. (New) A protein according to claim 31 wherein the amino acid residue corresponding to residue 105 in *Photinus pyralis* luciferase and to residue 106 of *Luciola mingrellica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis* gene luciferase is valine,

44. (New) A protein according to claim 31 which comprises a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 234 in *Photinus pyralis* luciferase is glycine.

45. (New) A protein according to claim 31 which comprises a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 420 in *Photinus pyralis* luciferase is threonine.

46. (New) A protein according to claim 31 which comprises a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 310 in *Photinus pyralis* luciferase is arginine.

47. (New) A protein according to claim 31 wherein the amino acid at position corresponding to amino acid 354 of the *Photinus pyralis* luciferase (356 in *Luciola luciferase*) is other than glutamate.

48. (New) A protein according to claim 47 wherein the amino acid at position

corresponding to amino acid 354 of the *Photinus pyralis* luciferase (356 in Luciola luciferase) is lysine or arginine.

49. (New) A protein according to claim 31 wherein the amino acid at the position corresponding to amino acid 217 in Luciola luciferase (215 in *Photinus pyralis*) is a different hydrophobic amino acid.

50. (New) A protein according to claim 49 wherein the amino acid at The position corresponding to amino acid 217 in Luciola luciferase (215 in *Photinus pyralis*) is isoleucine, leucine or valine

51. (New) A nucleic acid which encodes a protein according to claim 31.

52. (New) A vector comprising a nucleic acid according to claim 51.

53. (New) A cell transformed with a vector according to claim 52.

54. (New) A cell according to claim 53 which is a prokaryotic cell.

55, (New) A cell according to claim 53 which is a plant cell.

56. (New) A plant comprising cells according to claim 55.

57. (New) In a bioluminescent assay which comprises a luciferase/luciferin reaction and detection of bioluminescence, wherein the improvement comprises contacting the protein according to claim 1 in said reaction.

58. (New) A kit comprising a protein according to claim 31.

59. (New) A kit according to claim 58 which further comprises luciferin.

60. (New) A recombinant protein (a) having luciferase activity (b) having an amino acid sequence having at least 60% identity to the amino acid sequence of *Photinus pyralis* luciferase and (c) being a mutated wild-type sequence; wherein, in the sequence of the recombinant protein, the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase is mutated as compared to the corresponding wild-type luciferase, such that the recombinant protein has enhanced thermostability as compared to the corresponding wild-type luciferase.

61. (New) The recombinant protein of claim 60 wherein the wild-type luciferase sequence is of a luciferase selected from the group consisting of *Photinus pyralis*, *Luciola mingrellica*, *Luciola cruciata*, *Luciola lateralis*, *Hotaria parvula*, *Pyrophorus plagiophthalmus*, *Lampyris noctiluca*, *Pyrocoelia miyako* and *Photuris pennsylvanica*.

62. (New) The recombinant protein of claim 60 wherein the wild-type luciferase sequence is the sequence of an enzyme a luciferase obtainable from *Photinus pyralis*, *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* enzyme.

63. (New) The recombinant protein of claim 60 wherein the sequence of the recombinant protein further contains at least one of the following mutations as compared to the corresponding wild-type luciferase:

(b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase or to residue 234 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase;

(c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase or to residue 297 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola lateralis* luciferase;

(d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to residue 16 of *Luciola mingrellica*, or 17 of *Luciola cruciata* or *Luciola lateralis*;

(e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to residue 37 of *Luciola mingrellica*, or 38 of *Luciola cruciata* or *Luciola lateralis*;

(f) the amino acid residue corresponding to amino acid residue 105 of the *Photinus pyralis* luciferase or to residue 106 of *Luciola mingrellica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis* gene;

(g) amino acid residue corresponding to amino acid residue 234 of the *Photinus*

*pyralis* luciferase or to residue 236 of *Luciola mingrellica*, *Luciola cruciata* or *luciola lateralis*;

(h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase or to residue 422 of *Luciferase mingrellica*, *Luciola cruciata* or *Luciola Lateralis*;

(i) amino acid residue corresponding to amino acid residue 310 of the *Photinus pyralis* luciferase or to residue 312 of *Luciola mingrellica*, *Luciola cruciata* or *Luciola Lateralis*

is different to the amino acid which appears in the corresponding wild type sequence and wherein the luciferase enzyme has increased thermostability as compared to an enzyme having the amino acid of the corresponding wild-type luciferase at this position.

64. (New) A nucleic acid which encodes a recombinant luciferase according to claim 60.

65. (New) The nucleic acid of claim 64 in a vector.

66. (New) The vector of claim 65 transformed in a cell.